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Nano Life Science Institute of Kanazawa University
Cancer Research Institute of Kanazawa University

Co-Organized by

Institute for Frontier Science Initiative of Kanazawa University
Kanazawa Association of Tumor Biologists

Symposium Website

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4th

Probing life at the nanoscale

NanoLSI Symposium

- Bio-imaging, sensing and manipulation for medical science -

International Symposium on Tumor Biology in Kanazawa2020

November 26-27, 2020

- Vanue -

Online

PROGRAMME

Session1

Molecular and Cellular Dynamics in Biological Regulation and Regenerative Medicine
Chair: Kunio MATSUMOTO (Professor / WPI-NanoLSI / CRI)

1	8:00 am-8:30 am (JST), Nov. 26 12:00 am-12:30 am (CET), Nov. 26 6:00 pm-6:30 pm (EST), Nov. 25	Simon SCHEURING Professor Weill Cornell Medicine	High-Speed Atomic Force Microscopy: A Forceful Tool for Molecular Biophysics
2	8:30 am-9:00 am (JST), Nov. 26 12:30 am-1:00 am (CET), Nov. 26 6:30 pm-7:00 pm (EST), Nov. 25	Yusuke MIYANARI Associate Professor WPI-NanoLSI / CRI	Toward understanding transcriptional events deep inside the chromatin jungle
3	9:00 am-9:30 am (JST), Nov. 26 1:00 am-1:30 am (CET), Nov. 26 7:00 pm-7:30 pm (EST), Nov. 25	Kenneth S. ZARET Professor University of Pennsylvania	Two-Parameter, Single-Molecule-Tracking Assessments Discriminate Diverse Regulatory Factor Behaviors in Chromatin
4	9:30 am-10:00 am (JST), Nov. 26 1:30 am-2:00 am (CET), Nov. 26 7:30 pm-8:00 pm (EST), Nov. 25	Takanori TAKEBE Professor Tokyo Medical and Dental University	Promise and Impact of Organoid Medicine

Session2

Chemistry-Driven Challenges: from Molecule to Nano/Microscale
Chair: Satoshi ARAI (Associate Professor / WPI-NanoLSI)

1	1:30 pm-2:00 pm (JST), Nov. 26 5:30 am-6:00 am (CET), Nov. 26 11:30 pm-12:00 am (EST), Nov. 25	Tomoki OGOSHI Professor Kyoto University / WPI-NanoLSI	Supramolecular Assemblies of Pillar[n]arenes for Molecular Separation, Artificial Water Channels and Biosensor Applications
2	2:00 pm-2:30 pm (JST), Nov. 26 6:00 am-6:30 am (CET), Nov. 26 12:00 am-12:30 am (EST), Nov. 26	Toshinori FUJIE Associate Professor (Lecturer) Tokyo Institute of Technology	Printed Nanofilm to Engineer Bioelectronic "Second Skin"
3	2:30 pm-3:00 pm (JST), Nov. 26 6:30 am-7:00 am (CET), Nov. 26 12:30 am-1:00 am (EST), Nov. 26	Etsuo SUSAKI Associate Professor The University of Tokyo	CUBIC-HistoVIsion: a versatile three-dimensional whole-organ/body staining and imaging based on electrolyte-gel properties of biological tissue
4	3:00 pm-3:30 pm (JST), Nov. 26 7:00 am-7:30 am (CET), Nov. 26 1:00 am-1:30 am (EST), Nov. 26	Susumu KITAGAWA Distinguished Professor Kyoto University	New Dimensions of Porous Coordination Polymers/ Metal-Organic Frameworks

Session3

Nano-Scale Approaches to Physiological and Pathological Phenomena
Chair: Toshio ANDO (Professor / WPI-NanoLSI)

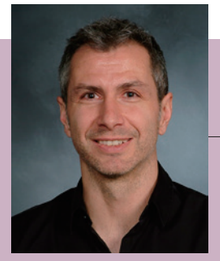
1	5:00 pm-5:30 pm (JST), Nov. 26 9:00 am-9:30 am (CET), Nov. 26 3:00 am-3:30 am (EST), Nov. 26	Nobuo NODA Laboratory Head Institute of Microbial Chemistry	Autophagy regulation by liquid-liquid phase separation
2	5:30 pm-6:00 pm (JST), Nov. 26 9:30 am-10:00 am (CET), Nov. 26 3:30 am-4:00 am (EST), Nov. 26	Noriyuki KODERA Professor WPI-NanoLSI	Single-molecule visualization of intrinsically disordered Rett syndrome protein, MeCP2 by high-speed Atomic Force Microscopy
3	6:00 pm-6:30 pm (JST), Nov. 26 10:00 am-10:30 am (CET), Nov. 26 4:00 am-4:30 am (EST), Nov. 26	Shinji WATANABE Associate Professor WPI-NanoLSI	Probing and characterizing nano-bio interfaces by scanning ion conductance microscopy
4	6:30 pm-7:00 pm (JST), Nov. 26 10:30 am-11:00 am (CET), Nov. 26 4:30 am-5:00 am (EST), Nov. 26	Victor SHAHIN Professor University of Münster	Facilitating nuclear delivery of pharmacological nanoparticles by interfering with the selective nuclear pore barrier

Session4

Imaging Approaches to Explore Cancer Biology
Chair: Masanobu OSHIMA (Professor / WPI-NanoLSI / CRI)

1	10:00 am-10:30 am (JST), Nov. 27 2:00 am-2:30 am (CET), Nov. 27 8:00 pm-8:30 pm (EST), Nov. 26	Keehoon JUNG Assistant Professor Seoul National University College of Medicine	Real-time intravital characterization of non-classical monocytes in cancers
2	10:30 am-11:00 am (JST), Nov. 27 2:30 am-3:00 am (CET), Nov. 27 8:30 pm-9:00 pm (EST), Nov. 26	Takeshi IMAMURA Professor Ehime University	Development of in vivo cancer imaging technique by advanced multi-photon laser excitation microscopy
3	11:00 am-11:30 am (JST), Nov. 27 3:00 am-3:30 am (CET), Nov. 27 9:00 pm-9:30 pm (EST), Nov. 26	Ann-Marie CHACKO Assistant Professor Duke-NUS Medical School	Developing novel probes for in vivo molecular PET imaging of cancer immunotherapy
4	11:30 am-12:00 pm (JST), Nov. 27 3:30 am-4:00 am CET), Nov. 27 9:30 pm-10:00 pm (EST), Nov. 26	Masanobu OSHIMA Professor WPI-NanoLSI / CRI	Polyclonal metastasis of colorectal cancer

ORAL PRESENTATION



Simon SCHEURING

Department of Anesthesiology. Department of Physiology and Biophysics
Weill Cornell Medicine. USA

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Research Interests

Membrane protein, Structure, Conformational changes, Atomic Force Microscopy, Force Spectroscopy

Education Professional Career

1997- 2001	PhD thesis: Summa cum laude, Biozentrum, University Basel, Switzerland
2001 - 2003	Postdocs: University Basel, Switzerland (2001), Institut Curie, Paris, France (2002, 2003)
2004 - 2007	CR2 INSERM – Researcher, Institut Curie, Paris, France
2001 - 2005	Habilitation (HDR), Université Pierre et Marie Curie, Paris 6, Jussieu, Paris, France
2007 - 2012	DR2 INSERM – Junior Research Director
2010 - 2011	Director INSERM U1006, Institut Curie, Paris, France
2012 - 2016	DR1 INSERM – Senior Research Director, INSERM
2012 - 2016	Director INSERM U1006, INSERM/Aix-Marseille Université, Marseille, France
2017- present	Professor, Weill Cornell Medicine, Anesthesiology, Physiology & Biophysics, NY, USA

Scientific Activities

1995 - present	Atomic Force Microscopy application and development

Honors

2019 - 2024	NIH Director's Pioneer Award (DP1)
2012 - 2017	ERC Starting/Consolidator Grant 2012
2006 - 2010	INSERM Avenir 2005 Program Awardee

Publications

1. Millisecond dynamics of an unlabeled amino acid transporter, Nature Communications, 2020, 11(5016), doi.org/10.1038/s41467-020-18811-z, TR Matin#, GR Heath#, GHM Huysmans, O Boudker & S Scheuring

2. Force-induced conformational changes in Piezo1, Nature, 2019, 573(7773):230–234, doi. 10.1038/s41586-019-1499-2, Y-C Lin#, YR Guo#, A Miyagi, J Levring, R MacKinnon* & S Scheuring

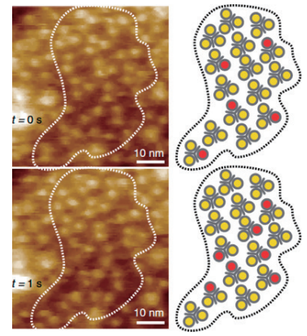
3. High-Speed AFM Height Spectroscopy (HS-AFM-HS): Microsecond dynamics of unlabeled biomolecules, Nature Communications, 2018, 9(1):4983. doi:10.1038/s41467-018-07512-3, GR Heath & S Scheuring

High-Speed Atomic Force Microscopy: A Forceful Tool for Molecular Biophysics

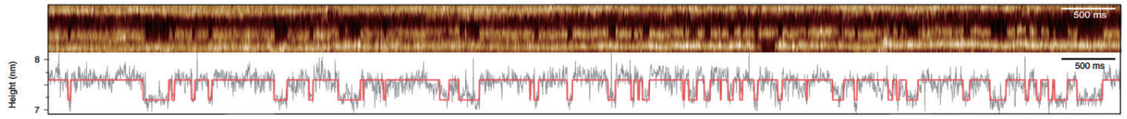
Simon SCHEURING

Department of Anesthesiology, Department of Physiology and Biophysics, Weill Cornell Medicine, USA

High-speed atomic force microscopy (HS-AFM)[1] is a powerful technique that provides dynamic movies of biomolecules at work. HS-AFM has the notable advantage that it permits to subject the proteins under investigation to environmental cues such as changes of pH, ions, ligands, temperature, light and force. This is particularly advantageous for the study of ion channels that respond to a wide range of stimuli [2], and transporters with substrate-dependent activities. To break current temporal limitations to characterize molecular dynamics using HS-AFM, we use HS-AFM line scanning (HS-AFM-LS) and HS-AFM height spectroscopy (HS-AFM-HS), a technique whereby we oscillate the HS-AFM tip at a fixed position and detect the motions of the molecules under the tip [3]. These methods allowed us to determine the dynamics of individual unlabeled amino acid transporters at millisecond temporal resolution [4].



HS-AFM frames and schematic of amino acid transporter GltPh in membrane.



Top: HS-AFM line scanning kymograph with 3.3ms temporal resolution of GltPh transport motion.
Bottom: Height/time trace and idealized state assignment (red).

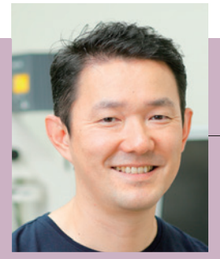
References

[1] A high-speed atomic force microscope for studying biological macromolecules, PNAS, 2001, 98(22):12468-12472, doi.org/10.1073/pnas.211400898, Toshio Ando, Noriyuki Kodera, Eisuke Takai, Daisuke Maruyama, Kiwamu Saito, and Akitoshi Toda

[2] Force-induced conformational changes in Piezo1, Nature, 2019, 573(7773):230–234, doi. 10.1038/s41586-019-1499-2, Yi-Chih Lin, Yusong R Guo, Atsushi Miyagi, Jesper Levring, Roderick MacKinnon & Simon Scheuring

[3] High-Speed AFM Height Spectroscopy (HS-AFM-HS): Microsecond dynamics of unlabeled biomolecules, Nature Communications, 2018, 9(1):4983. doi:10.1038/s41467-018-07512-3, George Heath & Simon Scheuring

[4] Millisecond dynamics of an unlabeled amino acid transporter, Nature Communications, 2020, 11(5016), doi.org/10.1038/s41467-020-18811-z, Tina R Matin, George R Heath, Gerard HM Huysmans, Olga Boudker & Simon Scheuring



Yusuke MIYANARI

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Cancer Research Institute, Kanazawa University, Japan

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Research Interests

Epigenetics, Stem cell, chromatin, transcription

Education

2006	PhD, Department of Human Tumor Viruses, Graduate School of Biostudies, Kyoto University

Professional Career

2006 - 2009	Postdoc, National Institute of Genetics (NIG), JAPAN
2009 - 2014	Postdoc, Institute of Genetics and Molecular and Cellular Biology (IGBMC), Strasbourg, FRANCE
2014 - 2020	Associate Professor (PI), National Institute of Basic Biology (NIBB), JAPAN
2020 - present	Associate Professor (PI), NanoLSI, Kanazawa University.

Scientific Activities

2001 - 2006	Studies on Hepatitis C virus
2006 - present	Epigenetic regulation of cell lineage allocation

Honors

2015	The Young Scientists' Prize, The Commendation for Science and Technology by the MEXT
2015	Takenaka Promotion Prize

Publications

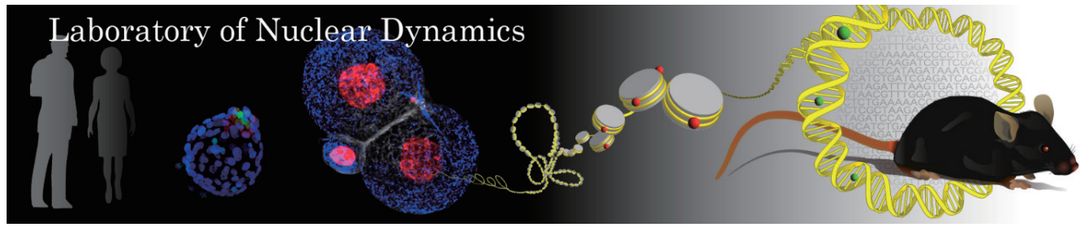
1. Miyanari Y, Torres-Padilla ME, Control of ground-state pluripotency by allelic regulation of Nanog, Nature, 483.470-473. 2012.
2. Miyanari Y*, Birling CZ. And Torres-Padilla ME*, Live visualization of chromatin dynamics using fluorescent TALEs, Nature Structural & Molecular Biology, 2013. *Corresponding authors
3. Kurihara M, Miyanari Y, et al., Genomic profiling of PML bodies reveals transcriptional regulation by PML bodies through the DNMT3A exclusion, Molecular Cell, 2020, Volume 78, Issue 3, 7 May 2020, Pages 493-505.e8.

Toward understanding transcriptional events deep inside the chromatin jungle

Yusuke MIYANARI
Nano Life Science Institute (WPI-NanoLSI),
Cancer Research Institute, Kanazawa University, Japan

Chromatin is organized in a non-random fashion within 3D nuclear space. During developmental processes, the nuclear architecture is dramatically reconstructed, resulting in the establishment of cell-type specific nuclear organization. Defects in structural components of the nucleus are responsible for developmental aberrations and several human diseases. Remodeling of the nuclear architecture leads to spatial arrangement of genes, which could affect genome functions including gene expression. We aim to reveal the role of chromatin dynamics in cell lineage-allocation by deciphering the molecular mechanisms underlying the remodeling of nuclear organization and their effects on developmental gene expression, using mouse early embryos and embryonic stem (ES) cells as model systems. We uncovered allelic regulation of key gene for reprogramming, Nanog, is crucial for cell-lineage allocation in early mouse embryos [1,2]. We also studied a role of PML bodies in transcriptional regulation in ES cells [3]. In this seminar, I will present our recent studies to show you how we tackle our questions.

The promyelocytic leukemia (PML) body is a phase-separated nuclear structure physically associated with chromatin, implying its crucial roles in genome functions. However, its role in transcriptional regulation is largely unknown. We developed APEX-mediated chromatin labeling and purification (ALaP) to identify the genomic regions proximal to PML bodies. We found that PML bodies associate with active regulatory regions across the genome and with ~300 kb of the short arm of the Y chromosome (YS300) in mouse embryonic stem cells. The PML body association with YS300 is essential for the transcriptional activity of the neighboring Y-linked clustered genes. Mechanistically, PML bodies provide specific nuclear spaces that the de novo DNA methyltransferase DNMT3A cannot access, resulting in the steady maintenance of a hypo-methylated state at Y-linked gene promoters. Our study underscores a new mechanism for gene regulation in the 3D nuclear space and provides insights into the functional properties of nuclear structures for genome function.

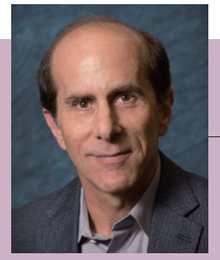


References

[1] Miyanari Y, Torres-Padilla ME, Control of ground-state pluripotency by allelic regulation of Nanog, Nature, 483.470-473. 2012.

[2] Miyanari Y*, Birling CZ. And Torres-Padilla ME*, Live visualization of chromatin dynamics using fluorescent TALEs, Nature Structural & Molecular Biology, 2013. *Corresponding authors

[3] Kurihara M, Miyanari Y, et al., Genomic profiling of PML bodies reveals transcriptional regulation by PML bodies through the DNMT3A exclusion, Molecular Cell, 2020, Volume 78, Issue 3, 7 May 2020, Pages 493-505.e8.



Kenneth S. ZARET

Institute for Regenerative Medicine (IRM)
University of Pennsylvania, USA

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Research Interests

Transcription factor-chromatin interactions, heterochromatin, cell reprogramming,

Education

1982	PhD, Biophysics, University of Rochester Medical School, Rochester, NY
1982-1985	Postdoctoral fellowship, Biochemistry and Biophysics, University of California, San Francisco. CA

Professional Career

1986- 1999	Professor, Cell, Molecular, and Developmental Biology, Brown University, RI
1999 - 2009	Senior Member, Program Leader, Cell and Dev. Biology Program, Fox Chase Cancer Ctr., PA
2009 - present	Professor and Director, IRM, Perelman School of Medicine, University of Pennsylvania, PA

Scientific Activities

1986 - present	Signaling control of cell fate in embryonic development, cell reprogramming
1993 - present	Discovery and mechanism of action of pioneer transcription factors
2012 - present	Biophysical methods, proteomics to understand compacted heterochromatin chromatin
2017 - present	Single-molecule-tracking to understand how transcription factors scan genomic chromatin

Honors

2002	Hans Popper Basic Science Award, Amer. Assoc. for the Study of Liver Diseases, ALF
2007	Elected as a Fellow of the American Assoc. for the Adv. of Science
2017	Stanley N. Cohen Biomedical Research Award, Univ. Penn.

Publications

1. Zaret, K.S. (2020) Pioneer transcription factors initiating gene network changes. **Annual Review of Genetics**, (e-pub: https://doi.org/10.1146/annurev-genet-030220-015007).

2. Iwafuchi, M., Cuesta, I., Donahue, G, Takenaka, N. Osipovich, A.B., Magnuson, M. A., Roder, H., Seeholzer, S.H., Santisteban, P., and Zaret, K.S. (2020) Gene network transitions in embryos depend upon interactions between a pioneer transcription factor and core histones. **Nature Genetics 52**, 418-427. PMID:32203463

3. Lerner, J., Gomez-Garcia, P.A., McCarthy, R., Liu, Z., Lakadamyali, M., and Zaret, K.S. (2020) Two-parameter mobility assessments discriminate diverse regulatory factors behaviors in chromatin. **Molecular Cell 79**, 418-5427. PMID: 32574554

Two-Parameter, Single-Molecule-Tracking Assessments Discriminate Diverse Regulatory Factor Behaviors in Chromatin

Kenneth S. ZARET
Institute for Regenerative Medicine, University of Pennsylvania, USA

Our laboratory is interested in the ways that transcription factors initiate cell fate changes in embryonic development, cell reprogramming, and human diseases such as cancer. We discovered that a certain class of gene regulatory proteins, which we called "pioneer transcription factors," initiate cell fate changes by their ability to target DNA sequences that are wrapped on a nucleosome at silent genes in cellular chromatin [1]. The initial targeting of nucleosomal DNA by a pioneer factor causes an exposure of the underlying nucleosome [2], thus allowing cooperating transcription factors, co-regulators, and ATP-dependent nucleosome remodelers to access the DNA and activate the targeted gene. Yet recent studies have shown that silent genes can exist in various states of compaction in chromatin, and pioneer factors have differential access to different types of silenced chromatin. To better understand these issues, our laboratory employed HALO-tags on diverse chromosomal proteins and transcription factors, which enabled single-molecule-tracking (SMT) of the individual molecules in real time, as the molecules diffuse in, or bind to, different chromatin domains in the nucleus of living cells. We began by using SMT of core histone proteins and quantified two parameters of molecular movement tracks over millisecond time scales [3]. We plotted the radius of confinement, which estimates the area in which the molecule performs its confined motions, against the average displacement, representing the average distance between subsequent steps in a motion track, over time. The two parameters, though generally positively correlated, present deviations that resolve five mobility types for core histones with distinct subnuclear localizations. As expected, the mobility of diverse heterochromatin proteins correlates with lower mobility chromatin, with notable differences that relate to particular biochemical features of the proteins. Upon assessing the mobility of diverse transcription factors, we found that pioneer factors with nucleosome binding ability can access the lowest mobility chromatin domains. Using well-defined mutations of the pioneer factor FOXA1, we found that nonspecific DNA/nucleosome binding and histone interactions are essential for scanning compacted chromatin. Our two-parameter SMT approach reveals how gene regulatory proteins scan the genome during cell fate changes.

References

[1] Zaret, K.S. (2020) Pioneer transcription factors initiating gene network changes. **Annual Review of Genetics**, (e-pub: https://doi.org/10.1146/annurev-genet-030220-015007).

[2] Iwafuchi, M., et al. (2020) Gene network transitions in embryos depend upon interactions between a pioneer transcription factor and core histones. **Nature Genetics 52**, 418-427. PMID:32203463

[3] Lerner, J., Gomez-Garcia, P.A., McCarthy, R., Liu, Z., Lakadamyali, M., and Zaret, K.S. (2020) Two-parameter mobility assessments discriminate diverse regulatory factors behaviors in chromatin. **Molecular Cell 79**, 418-5427. PMID: 32574554

Takanori TAKEBE

Institute of Research, Tokyo Medical and Dental University, Japan
Communication Design Center, Yokohama City University, Japan
Division of Gastroenterology, Hepatology and Nutrition and Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, USA

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Research Interests

Stem cell and developmental biology, organoid medicine

Education

2011	M.D., Yokohama City University School of Medicine
2019	Ph.D., Yokohama City University School of Medicine

Professional Career

2011-2013	Research Associate, Department of Regenerative Medicine, Yokohama City University
2013-2018	Associate Professor, Department of Regenerative Medicine, Yokohama City University
2015 - present	Assistant Professor, Division of Gastroenterology, Hepatology and Nutrition and Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, USA
2017 - present	Director of Commercial Innovation, Center for Stem Cell and Organoid Medicine (CuSTOM), Cincinnati Children's Hospital Medical Center, USA
2018 - present	Professor & Founding Director, Communication Design Center, Yokohama City University
2018 - present	Professor, Institute of Research, Tokyo Medical Dental University

Scientific Activities

2018 - present	Deputy to the Chairman, Japanese Society for Regenerative Medicine (JSRM)
2018 - present	Board of Directors, International Society for Stem Cell Research (ISSCR)

Honors

2020	NIH Director's New Innovator Award, Bethesda, USA
2018	JSPS Prize of The Japan Society for the Promotion of Science, Tokyo
2016	Robertson Investigator Award, New-York Stem Cell Foundation, NY

Publications

1. Koike H, Iwasawa K, Ouchi R , Maezawa M, Giesbrecht K, Saiki N, R-R, Ferguson A, Kimura M , Wendy T, Wells J, Zorn A, and **Takebe T**: Modeling human hepato-biliary-pancreatic organogenesis from the foregut-midgut boundary. ***Nature***, 574(7776):112-116, 2019.

2. **Takebe T***, Wells JM *: Organoids-By-Design. ***Science***, 364 (6444), 956-95, 2019. (* Correspondence)

3. Camp JG, Sekine K, Gerber T, Loeffler-Wirth H, Binder H, Gac M, Kanton S, Kageyama J, Damm G, Seehofer D, Belicova L, Bickle M, Barsacchi R, Okuda R, Yoshizawa E, Kimura M, Ayabe H, Taniguchi H, **Takebe T***, Treutlein B*: Multilineage communication regulates human liver bud self-organization from pluripotency. ***Nature***, 546, 533–534, 2017. (* Correspondence)

Promise and Impact of Organoid Medicine

Takanori TAKEBE
Institute of Research, Tokyo Medical and Dental University, Japan
Communication Design Center, Yokohama City University, Japan
Division of Gastroenterology, Hepatology and Nutrition and Division of Developmental Biology,
Cincinnati Children's Hospital Medical Center, USA

Organoids are multicellular structures that can be derived from adult organs or pluripotent stem cells. Early versions of organoids range from simple epithelial structures to complex, disorganized tissues with large cellular diversity. The current challenge is to engineer cellular complexity into organoids in a controlled manner that results in organized assembly and acquisition of tissue function. These efforts have relied on studies of organ assembly during embryonic development and have resulted in development of organoids with multilayer tissue complexity and higher order functions. For example, we show that antero-posterior interactions recapitulate the foregut and the midgut boundary in vitro, modeling the inter-coordinated specification and invagination of the human hepato-biliary-pancreatic system from human pluripotent stem cells. Coupled with patient-derived stem cells, my group studied the mechanisms of human hepatic diseases that includes viral hepatitis, steatohepatitis, recently extended to drug induced liver injury (DILI), wherein organoid modelled the clinical phenotype and genotype are correlated. Here I will summarize the next generation of organoid by design, and discuss its promise and impact to elucidate personalized disease mechanisms and understand drug reactions underlying individual variations in humans.

References

[1] Koike H, Iwasawa K, Ouchi R , Maezawa M, Giesbrecht K, Saiki N, R-R, Ferguson A, Kimura M , Wendy T, Wells J, Zorn A, and **Takebe T**: Modeling human hepato-biliary-pancreatic organogenesis from the foregut-midgut boundary. ***Nature***, 574(7776):112-116.

[2] Ouchi R, Togo S, Kimura M, Shinozawa T, Koido M, Koike H, Thompson W, Karns R, Mayhew C, McGrath PS, McCauley HA, Zhang RR, Lewis K, Hakozaki S, Ferguson A, Saiki N, Yoneyama Y, Takeuchi I, Mabuchi Y, Akazawa C, Yoshikawa HY, Wells JM, **Takebe T***: Modeling Steatohepatitis in Humans with Pluripotent Stem Cell-Derived Organoids. ***Cell Metabolism***, 30(2):374-384, 2019 (*Correspondence)

[3] Koido M, Kawakami E, Fukumura J, Noguchi Y, Ohori M, Nio Y, Nicoletti P, Aithal G, Daly, A, Watkins P, Anayama H, Dragan Y, Shinozawa T and **Takebe T***. Polygenic architecture informs potential vulnerability to drug-induced liver injury. ***Nature Medicine***, 2020. (* Correspondence), PMID: 32895570

[4] Shinozawa T , Kimura M , Yuqi C, Saiki N, Yoneyama Y, Ouchi R , Koike H , Koido M, Zhang R-R, Dunn A, Ferguson A, Togo S, Lewis K, Thompson W, Asai A , **Takebe T***: High-Fidelity Drug Induced Liver Injury Screen Using Human iPSC Liver Organoids. ***Gastroenterology***, in press.

Tomoki OGOSHI

Graduate School of Engineering, Kyoto University, Japan
Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan

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Research Interests

Supramolecular Chemistry, Molecular Recognition, Assembly, Organic Chemistry, Polymer Chemistry

Education

2000	B.S. Faculty of Engineering, Kyoto University
2002	M.S. Graduate School of Engineering, Kyoto University
2005	Ph.D. Graduate School of Engineering, Kyoto University (Supervisor: Prof. Yoshiki Chujo)

Professional Career

2005 - 2006	JSPS Research Fellow (PD): Osaka University (Supervisor: Prof. Akira Harada)
2006 - 2010	Assistant Professor at Kanazawa University
2010 - 2015	Associate Professor at Kanazawa University
2015 - 2019	Professor at Kanazawa University
2019 - present	Professor at Kyoto University
2019 - present	Specially Appointed Professor at Kanazawa University

Scientific Activities

2013 - 2017	JST-PRESTO Researcher : JST PRESTO program
2018 - present	JST-CREST Research Investigator

Honors

2019	Kao Academic Award
2016	Merck Banyu Lectureship Award / Banyu Chemist Award (MSD Life Science Foundation)
2016	Nozoe Memorial Award for Young Organic Chemists (JPOC)
2014	The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology (MEXT)
2013	Cram Lehn Pedersen Prize in Supramolecular Chemistry (RSC)
2012	The Chemical Society of Japan Award for Young Chemists (CSJ)

Publications

1. Ogoshi, T.; Kanai, S.; Fujinami, S.; Yamagishi, T.; Nakamoto, Y. J. Am. Chem. Soc. 2008, 130, 5022-5023.
2. Ogoshi, T.; Yamagishi, T.; Nakamoto, Y. Chem. Rev. 2016, 116, 7937–8002.
3. Ogoshi, T.; Sueto, R.; Yagyu, M.: Kojima, R.: Kakuta, T.: Yamagishi, T.: Doitomi, K.: Tummanapelli, A. K.: Hirao, H.: Sakata, Y.: Akine, S.: Mizuno, M. Nat. Commun. 2019, 10, 479.
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Supramolecular Assemblies of Pillar[n]arenes for Molecular Separation, Artificial Water Channels and Biosensor Applications

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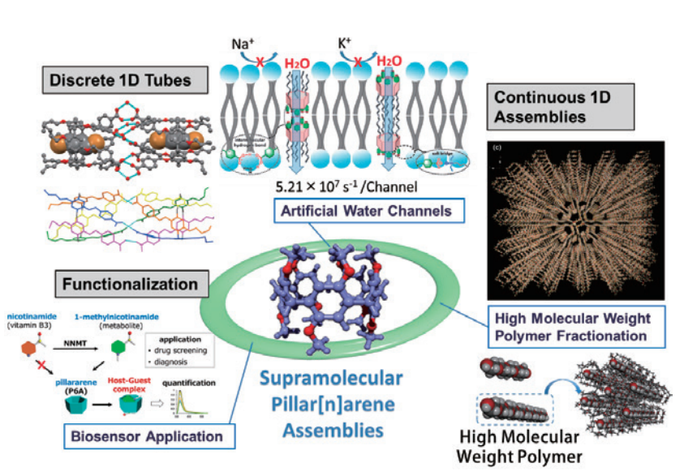
Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Japan

Macrocyclic compounds play a major role in supramolecular chemistry because of their beautiful shape, nano-scale size and molecular recognition ability. Numerous supramolecular architectures have been constructed and studied as new components of materials as well as entities related to biological structural formation and functions using various macrocyclic hosts.
In 2008, we reported a new class of macrocyclic hosts named “pillar[n]arenes”.[1,2] They have unique symmetrical pillar structures due to their para-bridge linkage. Based on the pillar-shaped structure, we have produced molecular scale porous materials by assembly of pillar[n]arenes, Due to the pillar-shaped structure, pillar[n]arenes mainly form one-dimensional (1D) channel structures in crystal state. We discovered that these 1D channel assemblies formed complexes selectively with high mass fraction of polymers when immersed in polymers with high polydispersity.[3] We have also contacted discrete 1D tubular assemblies using pillar[5]arenes with different rims and applied these discrete tubes for highly selective artificial water channels.[4] Biosensor applications of functionalized pillar[6]arenes for quantitative detection of a vitamin metabolite in crude biological samples are also reported.[5]



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Research Interests

Polymer chemistry, Biomaterial, Printed electronics, Wearable & implantable device, BioRobotics

Education

2009	Doctor of Engineering, Department of Life Science and Medical Bioscience, Waseda University

Professional Career

2010- 2012	Postdoctoral Research Fellow, Italian Institute of Technology
2012- 2013	Research Associate, Advanced Institute for Materials Research (AIMR), Tohoku University
2013- 2018	Assistant Professor, Department of Life Science and Medical Bioscience, School of Advanced Science and Engineering / Waseda Institute for Advanced Study, Waseda University
2015- 2019	Researcher, Precursory Research for Embryonic Science and Technology (PRESTO), JST
2018 - present	Associate Professor (Lecturer), School of Life Science and Technology, Tokyo Institute of Technology / Leading Initiative for Excellent Young Researchers (LEADER), JSPS

Scientific Activities

2005 -2015	Construction of polymeric nanosheets and their biomedical application
2015 - present	Integration of nano, bio, electronics for wearable and implantable device

Honors

2019	Biomaterials Science Emerging Investigators (Royal Society of Chemistry Journal)
2018	The Young Scientists' Prize for the Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology (MEXT), Japan
2017	The Award for Young Investigator of Japanese Society for Biomaterials; Japanese Society for Biomaterials

Publications

1. Suematsu, Y., Tsai, Ya An., Takeoka, S., Franz, C.M., Arai, S. Fujie, T. J. Mater. Chem. B, 8, 6999-7008 (2020).
2. Yamagishi, K., Nakanishi, T., Mihara, S., Azuma, M., Takeoka, S., Kanosue, K., Nagami, T., Fujie, T. NPG Asia Mater., 11, 80 (2019).
3. Yamagishi, K., Kirino, I., Takahashi, I., Amano, H., Takeoka, S., Morimoto, Y., Fujie, T. Nat. Biomed. Eng., 3, 27-36 (2019).

Printed Nanofilm to Engineer Bioelectronic "Second Skin"

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Integration of flexible electronics into the living system is expected for advancing medical diagnostics and therapeutics. Such devices should be conformable to the physical and mechanical environment of our body, in which acquired biosignals should be wirelessly transmitted to external device for health-care management. In this regard, we have envisaged ultra-flexible wearable and implantable devices based on the polymer nanosheet technology. The polymer nanosheet shows tens- to hundreds-of-nanometer thickness close to the scale of biomembranes [1], in which various types of polymers (e.g., biodegradable polymers, conductive polymers, and elastomers) can be formed into the ultra-thin structure by spincoating, layer-by-layer and gravure coating processes. The free-standing nanosheet showed flexible and adhesive properties derived from ultra-small flexural rigidity (< 10-2 nN m). In this talk, nanosheet-based devices (namely, printed nanofilms) are introduced by combining nanosheet and printing technologies with variety of unique inks (Fig. 1) [2]. The printed nanofilm has been utilized as a "second skin" to engineer functional bioelectronic interface, such as ultra-conformable bioelectrodes for sports science [3] and plant biology [4], and wirelessly-powered, implantable optoelectronics for photodynamic cancer therapy [5].

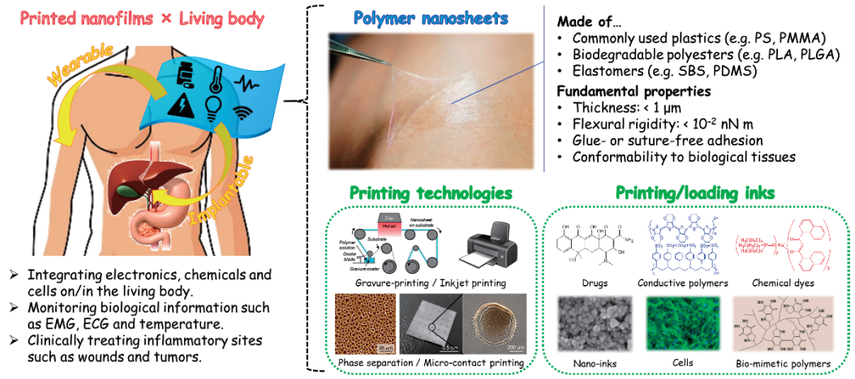

Fig 1. Printed nanofilms based on nanosheet technology. (Partially reproduced from Ref. 2.)

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Etsuo A. SUSAKI

Department of Systems Pharmacology, Graduate School of Medicine, The University of Tokyo, Japan

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Research Interests

Multicellular Systems Biology, Aging, Disease model, Tissue clearing, 3D imaging

Education

2002	Medical doctor, Faculty of Medicine, Kyushu University
2007	Doctor of Medicine, Graduate School of Medicine, Kyushu University

Professional Career

2006 - 2010	Post-doc, Medical Institute of Bioregulation, Kyushu University
2010 - 2013	Post-doc (JSPS PD/RIKEN SPR), RIKEN CDB
2013 - present	Faculty staff (2019- associate prof.), Graduate School of Medicine, The University of Tokyo

Scientific Activities

2002 -2010	Research on cell cycle/cell activity states and their molecular mechanisms
2010 - present	Development of cell-omics and high-throughput genetics technology

Honors

2017	The Young Scientists' Prize of The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology
2019	Selected award of PRESTO "Single-cell" area, Japan Science and Technology Agency

Publications

1. Susaki EA et al., "Versatile whole-organ/body staining based on electrolyte-gel properties of biological tissues", Nature Communications 11 (2020) 1982.
2. Susaki EA and Ueda HR. "Whole-body and Whole-Organ Clearing and Imaging Techniques with Single-Cell Resolution: Toward Organism-Level Systems Biology in Mammals", Cell Chemical Biology 23 (2016) 137-157.
3. Susaki EA et al., "Whole-Brain Imaging with Single-Cell Resolution Using Chemical Cocktails and Computational Analysis", Cell 157 (2014) 726-739.

CUBIC-HistoVIsion: a versatile three-dimensional whole-organ/body staining and imaging based on electrolyte-gel properties of biological tissue

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The recent development of various tissue clearing and three-dimensional (3D) imaging methods, including our CUBIC pipeline [1-2], allowed the comprehensive observation of the whole organ/body with cellular resolution or more. However, in the long history of histology, whole-organ/body 3D staining and imaging have been challenging due to the difficulty of adequate penetration of stains and antibodies. Even a small dye occasionally exhibits resistance to penetration, implying a complex physicochemical environment in the staining system.

In this presentation, we will introduce a versatile whole-organ/body staining and imaging protocol named CUBIC-HistoVIsion [3]. To dissect the complex physicochemical environment, we first conducted a precise characterization of biological tissue as an electrolyte gel. Then, we experimentally evaluated a broad range of 3D staining conditions by using a simplified tissue-mimicking artificial electrolyte gel. The combination of essential conditions allowed a bottom-up design of efficient 3D staining protocol which could uniformly label adult whole mouse brains, an adult marmoset hemisphere, a ~1 cm3 tissue block of adult human postmortem cerebellum, and an infant whole marmoset body with dozens of antibodies and cell-impermeant nucleic acid stains. We also demonstrate that our protocol enabled structural and functional neural circuit identification and analysis with Rabies virus tracing and whole-brain c-Fos immunostaining. The CUBIC-HistoVIsion offers advanced opportunities for organ- and organism-scale histological analysis of multicellular systems in the brain and body.

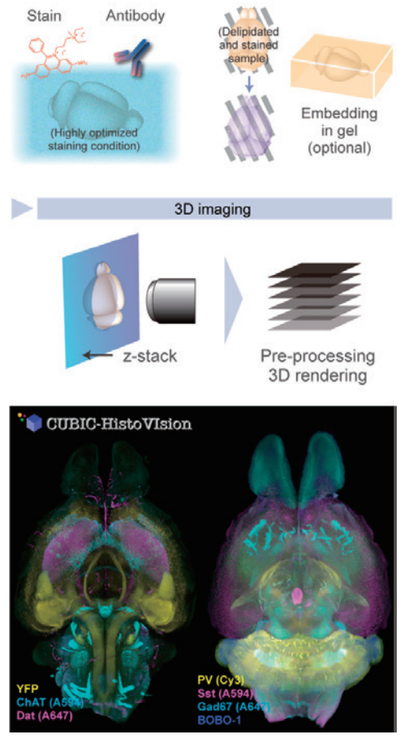

Fig. 1 Overview of CUBIC-HistoVIsion and representative 3D imaging data of whole mouse brains.

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[2] Susaki et al. Nature Protocols 10: 1709-1727 (2015)
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Kyoto University Institute for Advanced Study (KUIAS), Japan

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Research Interests

Coordination Chemistry, Porous Materials, Porous Coordination Polymers (PCPs), Metal-organic Frameworks (MOFs)

Education

1971 - 1974	Kyoto University, Undergraduate course, Hydrocarbon Chemistry
1975 - 1979	Kyoto University, Graduate School, Hydrocarbon Chemistry, PhD

Professional Career

2013 - present	Director, Institute for Integrated Cell-Material Sciences, Kyoto University
2017 - present	Distinguished Professor (KUIAS)
2020 - present	Deputy Director-General (KUIAS)

Scientific Activities

2013 - 2018	Project Leader of ACCEL, JSPS
2013 - 2018	Project Leader of Grant-in-Aid for Scientific Research for Specially Promoted Research "Chemistry of Hierarchical Coordination Space"
2012 - 2018	Project Leader of ACT-C, JSPS "Synthesis of porous coordination-based catalyst for conversion of carbon dioxide to methanol"

Honors

2017	Chemistry for the Future Solvay Prize
2018	Grand Prix of the Fondation de la Maison de la Chimie
2016	Japan Academy Prize
2011	The Medal with Purple Ribbon (The Japanese Government)

Publications

1. "Design and control of gas diffusion process in a nanoporous soft crystal", C.Gu, et al., Science, 2019, 363, 387-391.
2. "Highly responsive nature of porous coordination polymer surfaces imaged by in situ atomic force microscopy", N.Hosono, et al., Nature Chemistry, 2018, 11, 109-116.
3. "Self-Accelerating CO Sorption in a Soft Nanoporous Crystal", H.Sato, et al., Science, 2014, 343, 167-170.
4. "Localized cell stimulation by nitric oxide using a photoactive porous coordination polymer platform", S.Diring, et al., Nature Commun. 2013, 4, 2684-2691.

New Dimensions of Porous Coordination Polymers/ Metal-Organic Frameworks

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Kyoto University Institute for Advanced Study (KUIAS), Japan

The recent advent of porous coordination polymers (PCPs) or metal-organic frameworks (MOFs) as new functional microporous materials, have attracted the attention of chemists and physicists due to not only scientific but also application interest in the creation of unprecedented regular nano-sized spaces and in the finding of novel phenomena [1]. For the sake of consistency, we will refer to these porous materials as MOFs. Beyond the robust frameworks of MOFs, we discovered the essential attribute of MOFs that is porous structural flexibility, dissimilar to the conventional porous materials. Porous crystals with soft properties are collectively named "soft porous crystal (SPC)", and flexible MOFs are a class of SPC [2]. Flexible MOFs have a great potential for gas science & technology [3,4], focusing on energy and environmental, and bio active gases. High-efficiency separation technology, different from conventional ways, is essential for low-energy separation of gas resources, flue gases, air, pollutant gases and other industrial materials. Regulation of physiological functions of cells and tissues by spatiotemporally controlled release of biologically active gases is also important[5]. The future aspects will also be discussed [6,7].

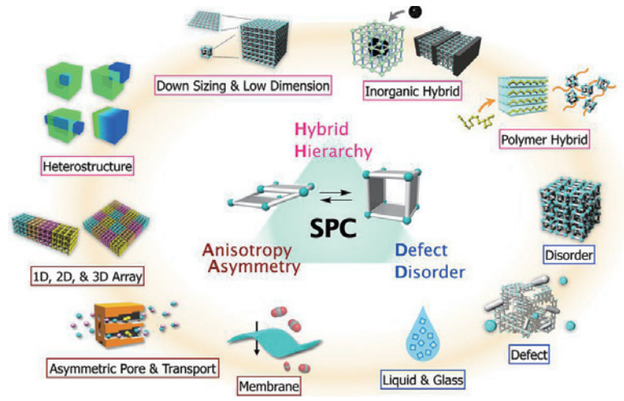

Figure 1. Different aspects of MOFs obtained from SPCs[7].

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[7] S.Kitagawa, et al.,Angew. Chem.Int. Ed., 2020, 59, 15325. Review article

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Research Interests

Autophagy, Phase Separation, Structural Biology, In Vitro Reconstitution

Education

2001 | Ph. D., Graduate School of Pharmaceutical Sciences, The University of Tokyo

Professional Career

2001 - 2005 | Postdoctoral Fellow, Hokkaido University
2005 - 2007 | Research Associate, Hokkaido University
2007 - 2011 | Lecturer with Tenure, Hokkaido University
2011 - 2017 | Chief Researcher, Institute of Microbial Chemistry
2017 - present | Laboratory Head, Institute of Microbial Chemistry

Scientific Activities

2002 - present | Structural biology of autophagy
2016 - present | In vitro reconstitution of autophagy and phase separation

Honors

2012 | Young Investigator Award, The Japanese Biochemical Society

Publications

1. Y. Fujioka, J. M. Alam, D. Noshiro, K. Mouri, T. Ando, Y. Okada, A. I. May, R. L. Knorr, K. Suzuki, Y. Ohsumi, N. N. Noda, “Phase separation organizes the site of autophagosome formation”, Nature, 578 (2020) 301-305.

2. A. Yamasaki, J. M. Alam, D. Noshiro, E. Hirata, Y. Fujioka, K. Suzuki, Y. Ohsumi, N. N. Noda, “Liquidity is a critical determinant for selective autophagy of protein condensates”, Mol. Cell, 77 (2020), 1163-1175.

3. T. Osawa, T. Kotani, T. Kawaoka, E. Hirata, K. Suzuki, H. Nakatogawa, Y. Ohsumi, N. N. Noda, “Atg2 mediates direct lipid transfer between membranes for autophagosome formation”, Nat. Struct. Mol. Biol., 26 (2019), 281-288.

Autophagy regulation by liquid-liquid phase separation

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Laboratory of Structural Biology, Institute of Microbial Chemistry, Japan

Autophagy is an intracellular degradation system that involves de novo generation of autophagosomes, which sequester cytoplasmic materials and deliver them to the lysosome/vacuole for degradation. When autophagy is induced by starvation, autophagy-related (Atg) proteins gather to form the pre-autophagosomal structure (PAS), which functions as a place for generating bulk autophagosomes that randomly sequester cytoplasmic materials. On the other hand, there exist selective types of autophagy termed “selective autophagy”, during which autophagosome generation proceeds on the specific large cargo that includes membrane-bound and membraneless organelles. Recently, we noticed that the PAS is a liquid droplet of Atg proteins that is formed by liquid-liquid phase separation (LLPS) [1]. LLPS of the PAS is strictly regulated by the phosphorylation state of Atg13, an intrinsically disordered protein. Upon starvation, Atg13 is dephosphorylated, which links Atg17 dimers to each other to form a large assemblage [2]. In vitro mixing of Atg13 with Atg17 resulted in the formation of liquid droplets, and point mutations in Atg13 or Atg17 that impaired the droplet formation in vitro also impaired the PAS formation in vivo, suggesting that the PAS is formed by the LLPS of the Atg13-Atg17 complex. High-speed atomic force microscopy observation of the Atg13-Atg17 droplets revealed randomly arranged, mobile Atg17 molecules inside the droplets, supporting the liquid-like feature of the droplets [1]. Aminopeptidase I (Ape1) is known to be a specific cargo for selective autophagy. We noticed that Ape1 undergoes LLPS to form Ape1 droplets both in vitro and in vivo [3]. Mutations that impair LLPS or solidify the Ape1 droplets impaired the selective autophagy of Ape1, suggesting that the liquid-like feature of Ape1 droplets is important for selective autophagy. In vitro reconstitution experiments revealed that liquid-like Ape1 droplets formed by LLPS, but not Ape1 aggregates that lost the liquidity by a mutation, were selectively sequestered by a membrane coated with Atg proteins. These data suggest that LLPS can be a general mechanism for regulating both bulk and selective autophagy.

References

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[2] H. Yamamoto, Y. Fujioka, S. W. Suzuki, D. Noshiro, H. Suzuki, C. Kondo-Kakuta, Y. Kimura, H. Hirano, T. Ando, N. N. Noda, Y. Ohsumi, “The intrinsically disordered protein Atg13 mediates supramolecular assembly of autophagy initiation complexes”, Dev. Cell, 38 (2016) 86-99.

[3] A. Yamasaki, J. M. Alam, D. Noshiro, E. Hirata, Y. Fujioka, K. Suzuki, Y. Ohsumi, N. N. Noda, “Liquidity is a critical determinant for selective autophagy of protein condensates”, Mol. Cell, 77 (2020), 1163-1175.

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Research Interests

biophysics, molecular motors, cytoskeletons, atomic force microscopy

Education

2001. Mar	B. Sci., Dept. of Phys, Fac. of Sci., Kanazawa Univ.
2003. Mar	M. Sci., Div. of Math. & Phys. Sci., Grad. Sch. Nat. Sci. & Tech., Kanazawa Univ.
2005. Sep	Ph.D., Div. of Basic Sci., Grad. Sch. Nat. Sci. & Tech., Kanazawa Univ.

Professional Career

2005 - 2010	Research Fellow (DC2 & PD), JSPS and Postdoctoral Fellow, CREST, JST
2010 - 2018	Assistant Professor & Associate Professor, Bio-AFM Frontier Research Center, Institute of Science and Engineering, Kanazawa University
2013 - 2017	PRESTO Researcher, JST
2018 - present	Professor, WPI-NanoLSI, Kanazawa University

Scientific Activities

2000 - 2008	Development of high-speed atomic force microscopy (HS-AFM)
2008- present	Biological application studies using HS-AFM and improvement of HS-AFM

Honors

2017	JSPS Prize
2013	Prize for Science and Technology (Development Category), The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology
2012	Young Researcher's Nanoprobe Technology Prize (JSPS Nanoprobe Technology 167)

Publications

1. **Kodera N**, Noshiro D, Dora SK, …, Longhi S, Ando T, "Structural and dynamics analysis of intrinsically disordered proteins by high-speed atomic force microscopy", ***Nat. Nanotech***. (in press).

2. Ngo KX, **Kodera N**, Katayama E, Ando T, Uyeda TQ, "Cofilin-induced unidirectional cooperative conformational changes in actin filaments revealed by high-speed atomic force microscopy", ***eLife 4***, 04806 (2015).

3. **Kodera N**, Yamamoto D, Ishikawa R, Ando T, "Video imaging of walking myosin V by high-speed atomic force microscopy", ***Nature 468***, 72-76 (2010).

Single-molecule visualization of intrinsically disordered Rett syndrome protein, MeCP2 by high-speed Atomic Force Microscopy

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High-speed atomic force microscopy (HS-AFM) directly visualizes biological molecules in action at nanometer spatial and sub-second temporal resolution. The unique performance of HS-AFM has been demonstrated by direct observations of many protein systems [1]. Importantly, HS-AFM can even resolve thin and flexible features of intrinsically disordered regions (IDRs), single polypeptide chains with height of ~0.5 nm, in a protein that could not be analyzed by any techniques at single molecule level. In some cases, dynamic structural transitions in IDRs between ordered- and disordered-conformations can be directly visualized [2-4].

Here, we applied HS-AFM to Methyl-CpG binding protein 2 (MeCP2). MeCP2 is a chromatin regulatory protein essential for brain development and activity in vertebrates. Specific missense and nonsense mutations in MeCP2 lead to the neurodevelopmental disorder, Rett syndrome (RTT). HS-AFM demonstrated that MeCP2 transitions between a fully extended dumbbell-like structure with the methyl DNA binding domain (MBD) and C-terminal domain (CTD) at the extremities, and a compact structure where the MBD and CTD interact in cis. The MBD within the full length protein equilibrates between unfolded and well folded states. MBD–CTD interactions stabilize the MBD in its folded state and are essential for MeCp2 plasticity (Fig. 1). The R106W, R133C, F155S and T158M RTT mutations all showed aberrant MBD dynamics compared to wild type. Furthermore, we observed sliding movements of MeCP2 along dsDNA, bridging two adjacent dsDNAs and DNA condensations by MeCp2. These results would gain insight into the molecular basis of RTT [5].

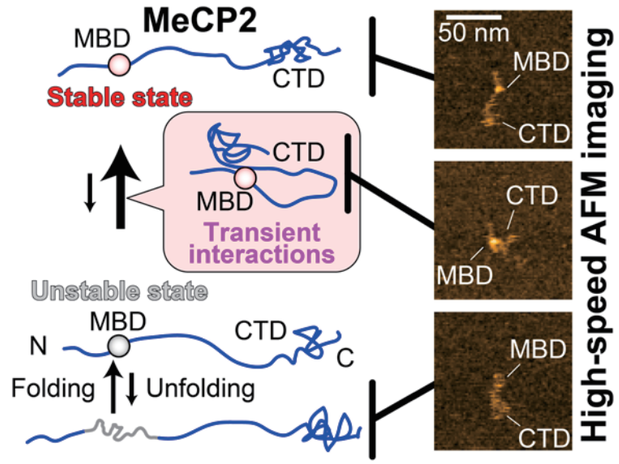

Fig.1 HS-AFM images and schematics showing that MBD-CTD interactions stabilize the MBD in its folded state.

References

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[2] M. Hashimoto, N. Kodera, Y. Tsunaka, M. Oda, M. Tanimoto, T. Ando, K. Morikawa, and S. Tate, Biophys. J., 2013, 104, 2222-2234.

[3] N. Terahara, N. Kodera, T. Uchihashi, T. Ando, K. Namba, and T Minamino, Sci. Adv., 2017, 3, eaao4119.

[4] N. Kodera, D. Noshiro, SK. Dora, …, S. Longhi, and T. Ando, Nat. Nanotech. (in press).

[5] N. Kodera, AA. Kalashnikova, …, T. Ando, and JC. Hansen, (in revision).

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Research Interests

Scanning Ion conductance microscopy, Nanoscience, Nanofluidics, Solid-liquid Interfaces,

Education

2006	Doctor of Science, Graduate School Science and Technology, Niigata University

Professional Career

2006	JST-CREST Postdoctoral Fellow, Niigata University
2006 - 2007	JST- SORST Postdoctoral Fellow, Niigata University
2007	Assistant Professor, Department of Physics, Tohoku University
2007 - 2009	Assistant Professor, Center for the Advancement of Higher Education, Tohoku University
2009 - 2012	JST-ERATO Postdoctoral Fellow, Tohoku University
2012 - 2017	Assistant Professor, Bio-AFM Frontier Research Center, Kanazawa University
2017 - 2020	Assistant Professor, Nano Life Science Institute, Kanazawa University
2020 - present	Associate Professor, Nano Life Science Institute, Kanazawa University

Scientific Activities

2004 - 2012	Spin physics and quantum transport in strongly correlated electron systems.
2012 - present	Instrumentation and applications of scanning ion conductance microscopy.

Publications

1. K. Shigyou, L. Sun, R. Yajima, S. Takigaura, M. Tajima, H. Furusho, Y. Kikuchi, K. Miyazawa, T. Fukuma, A. Taoka, T. Ando, S. Watanabe, "Geometrical Characterization of Glass Nanopipettes with Sub-10-nm Pore Diameter by Transmission Electron Microscopy" Anal. Chem. accepted.
2. S. Watanabe, S. Kitazawa, L. Sun, N. Kodera, and T. Ando, "Development of High-Speed Ion Conductance Microscopy" Rev. Sci. Instrum. 90(12), 123704 (2019).
3. L. Sun, K. Shigyou, T. Ando, and S. Watanabe, "Thermally Driven Approach to Fill Sub-10-nm Pipettes with Batch Production" Anal. Chem. 91, 14080, (2019).
4. S. Watanabe, and T. Ando, "High-speed XYZ-nanopositioner for scanning ion conductance microscopy" Appl. Phys. Lett. 111, 113106, (2017).

Probing and characterizing nano-bio interfaces by scanning ion conductance microscopy

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Scanning probe techniques with a glass capillary have been widely exploited in the characterization of physical, chemical, and biological properties of nano-bio interfaces. Among these techniques, scanning ion conductance microscopy (SICM) has played an essential role in the visualization of live cell surfaces with nanometer-scale resolution. SICM uses an electrolyte-filled glass pipette as a probe to detect an ion current passing through an aperture of the pipette tip. The ion current variation depending on the distance between the pipette tip and sample surface reflects various local information of sample surfaces under a liquid environment, such as geometry, surface charge, and mechanical properties of the sample (Fig. 1). However, a longstanding drawback of SICM is an insufficient spatiotemporal resolution. We have devoted to improve the insufficiency of SICM to realize wide-range applicability of SICM for the investigation of nano-bio interfaces [1-4]. Here we show recent progress of our improvements and applications using our instrument. We developed a tip-scan-type high-speed SICM scanner with a large stroke [1], an active damping control method to reduce unwanted vibrations due to the driving of the scanner [2], and the signal enhancement method with an ion concentration gradient produced in the vicinity of the tip [2]. These improvements allow us not only to visualize dynamic biological processes occurring in soft cell surfaces with high roughness but also to map local mechanical properties of live cell surface.

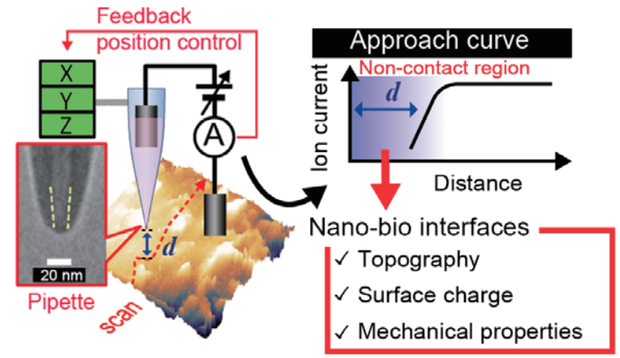

Fig. 1: Probing nano-bio interfaces by SICM

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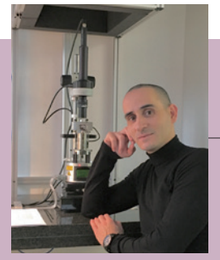

Victor SHAHIN

Institute of Physiology II, University of Muenster Medical Faculty, Germany

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Research Interests

Atomic Force Microscopy, Nanomedicine, Nanotechnology, Physiology, Cancer Physiology

Education

2002	Doctor of Pharmacy and Physiology, Pharmacy and Medical Faculties of Münster University, Germany

Professional Career

2002-2006	Postdoc, Medical Faculty of Münster/Germany, and University of Cambridge UK
2006-2015	Associate Prof. and group leader, Inst. of Physiology II, Medical Faculty of Münster Univ.
2015 - present	Prof. for Physiology, Inst. of Physiology II, Medical Faculty of Münster Univ.

Honors

2011	Rolf-Dierichs-Stiftung, 'Innovative Medical Research' award at the Medical Faculty, Münster
2018	Distinguished lecturer of the year award' Medical Faculty, Münster

Publications

1. Liashkovich, I., Rosso, G., Shahin, V., (2019). Nuclear envelope permeability barrier as a fast-response intracellular mechanostat. Advanced Science, 2019 Aug 29;6(21):1900709. doi: 10.1002/advs.201900709.

2. Azzam, I., Liashkovich, I., Luchtefeld, I., Kouzel IU, Shahin, V., (2019). Facilitating plasmid nuclear delivery be interfering with the selective nuclear pore barrier. Bioeng Transl Med. 2019 Jun 22;4(3):e10136. doi: 10.1002/btm2.10136.

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Facilitating nuclear delivery of pharmacological nanoparticles by interfering with the selective nuclear pore barrier

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Nuclear pore complexes (NPCs) are elaborate nano-transporters built from diverse proteins termed nucleoporins (Nups). They control all nucleocytoplasmic transport and form a stringent barrier between the cytosol and the nucleus. While selective nucleocytoplasmic transport enables translocation of macromolecules up to striking sizes approaching megadalton-scale, the upper cutoff for unselective diffusion is at 40 kDa. Elevating the cutoff is of particular importance for nuclear delivery of therapeutic nanoparticles that are destined to act inside the nucleus. We study compounds that interfere with the biophysical and functional properties of NPC. The ultimate goal is to raise the upper NPC cutoff for passive nuclear delivery to a substantial degree, relevant for therapeutic nanoparticles. We present two different classes of compounds that significantly lower the stringency of the NPC barrier, by two distinct modes of action, interfering with either the NPC channel barrier or the NPC scaffold. Our ongoing research reveals that former class facilitates nuclear delivery of 5kbp pDNA in up to 10-20% of the tested cells, compared to no delivery at all in control conditions. We envisage that the various tested compounds of this class may serve as lead substances and usher in the design of potent new strategies to increase nuclear delivery of therapeutic nanoparticles.

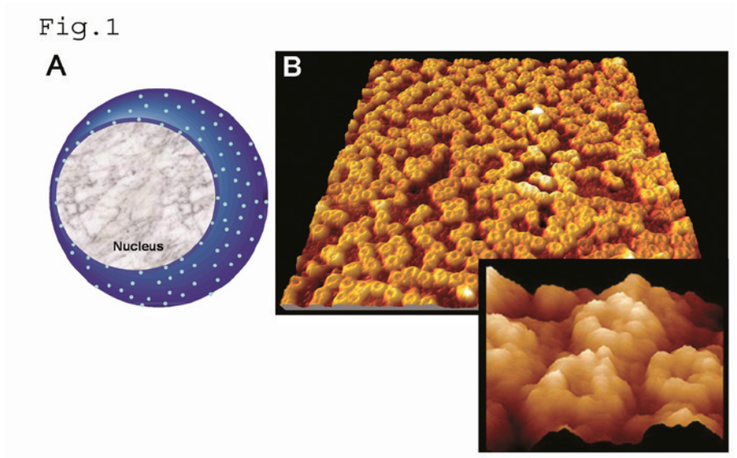

Fig. 1. A: Scheme of the cell nucleus. B: AFM images of the cytoplasmic face of the nuclear envelope (large image, 4 x 4 µm) and selected NPCs (magnification, bottom right, 280 x 280 nm) of Xenopus laevis oocytes.From: Shahin,V. (2006). The nuclear barrier is structurally and functionally highly responsive to glucocorticoids. BioEssays 28, 935-942.

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Research Interests

Cancer Immunology, In Vivo Imaging, Single-Cell Genomics, Vascular Biology, Drug Development

Education

2005	B.S. in Biological Sciences, KAIST, Korea
2010	Ph.D. in Biological Sciences and Biomedical Science & Engineering Program, KAIST, Korea

Professional Career

2010 -2013	Research Fellow at Wellman Center (In Vivo Imaging), Harvard Medical School / MGH
2013 -2018	Research Fellow at Steele Laboratories for Tumor Biology, Harvard Medical School / MGH
2018- present	Assistant Professor, Seoul National University

Honors

2018	Milstein Award, International Cytokine & Interferon Society (ICIS)
2018 -2027	Creative- Pioneering Researchers Program, Seoul National University
2017	Speaker Award, Gordon Research Conference - Angiogenesis
2016	Bristol-Myers Squibb (BMS) Award, Tumor Immunology and Immunotherapy Meeting
2016	AACR-GYRIG Scholar-in-Training Award, AACR Annual Meeting

Publications

1. Choo YW, Jeong J, Jung K. Recent advances in intravital microscopy for investigation of dynamic cellular behavior in vivo. BMB Reports, (2020).
2. Jeong J, Suh Y, Jung K. Context drives diversification of monocytes and neutrophils in orchestrating the tumor microenvironment. Frontiers in Immunology (2019).
3. Jung K. et al., Targeting CXCR4-dependent immunosuppressive Ly6C(low) monocytes improves antiangiogenic therapy in colorectal cancer. Proc Natl Acad Sci USA (2017).
4. Jung K. et al., Ly6C(lo) monocytes drive immunosuppression and confer resistance to anti-VEGFR2 cancer therapy. J Clin Invest (2017).

Real-time intravital characterization of non-classical monocytes in cancers

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Colorectal cancer (CRC) is the leading cause of cancer-related deaths worldwide. However, current anti-VEGF therapies for CRC provide limited survival benefit as tumors rapidly develop resistance to these agents.
We have developed a miniaturized confocal endomicroscopy technique for spontaneous CRC models in mice. We recently also established a novel abdominal imaging window. These unique systems enable us to monitor the CRC and its immune microenvironment longitudinally with a video-rate intravital multi-photon microscope. Using these in vivo imaging methods and CRC models, we have uncovered an immunosuppressive role for non-classical Ly6Clow monocytes that mediates resistance to anti-VEGFR2 treatment. We found that the chemokine CX3CL1 was upregulated in both human and murine tumors following the VEGF signaling blockade, resulting in recruitment of CX3CR1+ Ly6Clow monocytes into the tumor. We also found that treatment with VEGF-A reduced expression of CX3CL1 in endothelial cells. Intravital microscopy revealed that CX3CR1 is critical for Ly6Clow monocyte transmigration across the endothelium in tumors. Moreover, Ly6Clow monocytes recruit Ly6G+ neutrophils via CXCL5 and produce IL-10, which inhibits adaptive immunity. Preventing Ly6Clow monocyte or Ly6G+ neutrophil infiltration into tumors enhanced inhibition of tumor growth with anti-VEGFR2 therapy. Furthermore, we developed a gene therapy using a nanoparticle formulated with a siRNA against CX3CL1, which reduced Ly6Clow monocyte recruitment and improved outcome of anti-VEGFR2 therapy in mouse CRCs.
Taken together, we identified immunosuppressive non-classical Ly6Clow monocytes as key players in tumor resistance to anti-angiogenic therapy in CRCs. We also revealed molecular mechanisms underlying anti-angiogenic treatment resistance, suggesting potential immunomodulatory strategies to enhance the long-term clinical outcome of anti-VEGF therapies, proven by state-of-the-art in vivo imaging modalities.

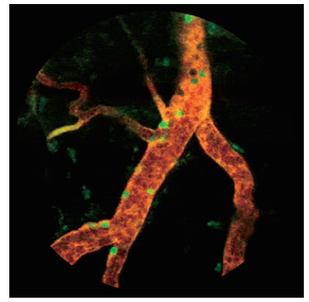

Fig. 1. Non-classical monocytes flow through blood vessels as they infiltrate tumors.

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[2] Jeong J, Suh Y, Jung K. Context drives diversification of monocytes and neutrophils in orchestrating the tumor microenvironment. Frontiers in Immunology (2019).
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[5] Jung K. et al., Endoscopic time-lapse imaging of immune cells in infarcted mouse hearts. Circ Res (2013).

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Research Interests

Bioimaging, Cancer biology, Bone biology, TGF-β signaling

Education

1980- 1987	MD. Graduate School of Medicine, Kagoshima University, Kagoshima, JAPAN.
1989- 1993	Ph.D. Graduate School of Medicine, Kagoshima University, Kagoshima, JAPAN.

Professional Career

1994- 1995	Assistant Professor, Graduate School of Medicine, Kagoshima University. Kagoshima, JAPAN.
1995- 1996	Postdoctoral Fellow, Ludwig Institute for Cancer Research. Uppsala, Sweden.
1996- 2004	Associate, Associate Member & Group Head, The JFCR Cancer Institute. Tokyo, JAPAN
2004- 2010	Department Director, The JFCR Cancer Institute. Tokyo, JAPAN
2010 - present	Professor, Ehime University Graduate School of Medicine. Ehime, JAPAN

Honors

2008	"JSBMR Distinguished Scientist Award" by the Society for Bone and Mineral Research
2000	"Incitement Award of the Japanese Cancer Association" by the Japanese Cancer Association

Publications

1. Inoue M et al. Rational Engineering of XCaMPs, a Multicolor GECI Suite for In Vivo Imaging of Complex Brain Circuit Dynamics. Cell. 177: 1346-60, 2019.
2. Yoon JH et al. Phosphorylation status determines the opposing functions of Smad2/Smad3 as STAT3 cofactors in TH17 differentiation. Nat Commun. 6: 7600, 2015
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Development of in vivo cancer imaging technique by advanced multi-photon laser excitation microscopy

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Ehime University Graduate School of Medicine, Japan

Recently, heterogeneity is one of the most popular keywords in cancer research. For instance, resistance to chemotherapy has long been thought to be attributed to the heterogeneity of cancer cells. However, it is difficult to prove this using only molecular biology. The easiest approach to answer this question is in vivo fluorescent imaging. This technique enables the researchers to observe the same cells in living animals for some time. In addition, this allow us to visualize the molecular activity, cellular function and microenvironment.

Fluorescent imaging technique is a promising technique, and has already been applied for in vitro experiments in cellular biology. Recently, there has been a growing interest in applying this fluorescent imaging technique to study different disease process and complex biology such as cancer in vivo. Particularly, the in vivo fluorescent imaging using various fluorophores and/or fluorescent proteins, in conjunction with appropriate microscopy, allows visualization of cell behavior as well as cell function in vivo.

In this talk, I will talk about applications of the fluorescent imaging systems to monitor cancer cell as well as tumor microenvironment such as angiogenesis in vivo. Using the in vivo fluorescent imaging system, we can visualize not only cell behavior but also cell function such as cell cycle and epithelial mesenchymal transition (EMT) [1, 2].

Finally, I would like to talk about a technological development of fluorescent imaging methods including two-photon microscopy and the application of the fluorescent imaging approaches to cancer research. Two-photon microscopy is a powerful method to investigate behavior and function of cells in deep tissues. Moreover, I would like to present the setup and application of infrared-(IR)-two-photon microscopy using excitation wavelengths above 1040 nm [3]. IR-two-photon microscopy enables us the application of red fluorescent proteins/dyes and deeper imaging compared with conventional multi-photon microscopy.

In vivo fluorescent imaging technique is now unravelling the cause and result of the heterogeneity among cancer cells and will pave a way to invent novel approaches for cancer treatment.

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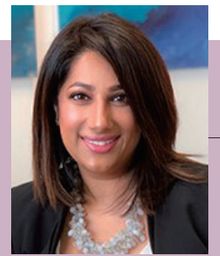

Ann-Marie CHACKO

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Research Interests

Molecular Imaging, In Vivo Diagnostics, Drug Delivery Systems, Cancer Immunotherapy, Clinical Translation

Education

2001	B.Sc. Honours Biochemistry, Bishop's University, Sherbrooke, QC, Canada
2003	M.Sc. Chemistry, University of Western Ontario, London, ON, Canada
2008	Ph.D. Pharmacological Sciences, University of Pennsylvania, Philadelphia (UPenn), PA, USA

Professional Career

2008- 2010	Postdoctoral Fellow, Pharmacology and Program in Targeted Therapeutics, Institute of Translational Medicine and Therapeutics (ITMAT), UPenn, Philadelphia, PA, USA
2011- 2012	Postdoctoral Fellow, Division of Clinical Molecular Imaging and Nuclear Medicine, Department of Radiology, UPenn, Philadelphia, PA, USA
2012- 2014	KL2 Fellow, Clinical and Translational Sciences, ITMAT, UPenn, Philadelphia, PA, USA
2012- 2014	Research Associate, Division of Clinical Molecular Imaging and Nuclear Medicine, Department of Radiology, UPenn, Philadelphia, PA, USA
2015 - present	Assistant Professor, Programme in Cancer and Stem Cell Biology, Duke-NUS Medical School, Singapore

Publications

1. Goggi JL, Hartimath SV, Hwang YY, Tan YX, Khanapur S, Boominathan R, Jiang L, Husaini AR, Cheng P, Yong FF Tan PW, Yuen TY, Jieu B, **Chacko AM**, Larbi A, Renia L, Johannes C, Robins, EG. Examining Immunotherapy Response Using Multiple Radiotracers. *Mol. Imaging Biol. Mol. Imaging Biol.* 2020; 22(4):993-1002.

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Developing novel probes for in vivo molecular PET imaging of cancer immunotherapy

Ann-Marie Chacko

Cancer and Stem Cell Biology Programme, Laboratory for Translational and Molecular Imaging, Duke-NUS Medical School, Singapore

Nuclear imaging technologies, such as positron emission tomography (PET), offers the potential to noninvasively visualise and quantify biomarkers at a cellular level as they occur in their native environment, and in real time. PET of the tumour immune microenvironment (TME) is a fairly nascent field of study, and is highly relevant in the era of Cancer Immunotherapy (CI), as it could provide a pivotal platform for validating treatment safety and efficacy for the number of CI combinations that are poised or already in clinical testing.

In this talk, I will outline our strategies to identify and validate prognostic and predictive biomarkers aimed to ultimately improve CI outcome. Specifically, I will highlight our preclinical efforts to track endogenous immune cell markers of metabolism and inflammation (18F-FDG-PET), proliferation (18F-FLT-PET), and cytotoxic T cell activity (18F-GZB-PET). We will also discuss strategies of taking probes beyond the preclinical space, towards the clinic. "Shedding light" on immune response and their modulation, will have important implications for assessing treatment response during therapeutic intervention trials in cancer, and in other immune-related diseases including emerging infectious disease.

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Cancer Research Institute, Kanazawa University, Japan

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Research Interests

Mouse genetics, organoid, microscopy imaging, microenvironment, metastasis

Education

1982- 1986	Faculty of Veterinary Medicine, Hokkaido University
1986- 1988	Hokkaido University Graduate School of Veterinary Medicine (MS)

Professional Career

1988- 1991	Staff Scientist, Chugai Pharmaceutical Co. Ltd.
1992- 1997	Staff Scientist, Banyu Tsukuba Research Institute (Merck)
1997- 1999	Research Associate, Merck Research Laboratories, USA
2000- 2005	Associate Professor, Kyoto University Graduate School of Medicine
2005 - present	Professor, Cancer Research Institute, Kanazawa University
2017 - present	Professor, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University

Scientific Activities

1992- 2005	Mouse genetics studies for chemoprevention of intestinal tumorigenesis
2005 - present	Mouse genetics and organoid research for gastrointestinal cancer malignant progression

Honors

2012	JCA-Mauverynay Award, Japanese Cancer Association
2013	Achievement Commendation of Kanazawa University, Kanazawa University
2015	The Commendation for Science and Technology, MEXT
2020	Ishikawa Television Award, Ishikawa TV Broadcasting Co. Ltd

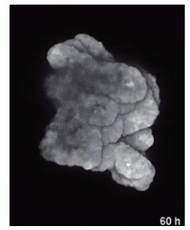
Publications

1. Nakayama M, Hong CP, Oshima H, Sakai E, Kim SJ, and Oshima M. Loss of wild-type p53 promotes mutant p53-driven metastasis through acquisition of survival and tumor-initiating properties. Nat Commun, 11: 2333. 2020.
2. Takeda H, Kataoka S, Nakayama M, Ali MAE, Oshima H, Yamamoto D, Park JW, Takegami Y, An T, Jenkins NA, Copeland NG, and Oshima M. CRISPR-Cas9 mediated gene knockout in intestinal tumor organoids provides functional validation for colorectal cancer driver genes. Proc Natl Acad Sci USA, 116: 15635-15644, 2019.
3. Oshima H, Kok SY, Nakayama M, Murakami K, Voon DC, Kimura T, and Oshima M. Stat3 is indispensable for damage-induced crypt regeneration but not for Wnt-driven intestinal tumorigenesis. FASEB J, 2019.

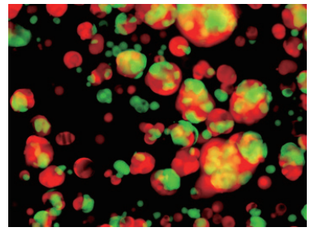
Polyclonal metastasis of colorectal cancer

Masanobu OSHIMA
Nano Life Science Institute (WPI-NanoLSI), Cancer Research Institute
Kanazawa University, Japan

Colorectal cancer (CRC) is a leading cause of cancer-related death globally, and the majority of cancer-related deaths are caused by metastasis. It is therefore important to predict metastatic ability of the primary cancers to establish a clinical strategy. The accumulation of driver mutations is responsible for development and malignant progression of colorectal cancer, and comprehensive genome research has identified driver genes that are frequently mutated in CRC. Using genetic mouse models, we have constructed mouse models and established intestinal tumor-derived organoids carrying various combinations of driver mutations, Apc (A), Kras (K), Tgfbr2 (T), Trp53 (P), and Fbxw7 (F). Comprehensive phenotype analyses revealed that AT and AP mutation combinations cause submucosal invasion, AKT and AKP combinations induce advanced malignant phenotype like epithelial-mesenchymal transition (EMT), and AKTP and AKTPF acquire highly metastatic ability from the spleen to liver [1-3], which is consistent with the established concept of multistep tumorigenesis based on Darwin evolution. However, a unique concept for metastasis, polyclonal metastasis, has recently been proposed. We therefore examined the mechanism of polyclonal metastasis by labeled the intestinal tumor-derived organoids with Venus and tdTomato. When non-metastatic (Non-M) cells are transplanted into the spleen with malignant metastatic (MM) cells, they form chimeric metastatic foci in the liver. Importantly, MM cells, but not Non-M cells, induce niche generation surrounding disseminated tumor clusters, and such microenvironment consisting of macrophages and αSMA-expressing cells promotes the colonization of Non-M cells. These results indicate that non-metastatic cells can metastasize via the polyclonal mechanism using the microenvironment that is generated by malignant cells.



AKTP organoids



AKTP and AP chimeric spheroids
for spleen transplantation

References

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Memo